INHIBITION OF CA 125, A NOVEL HIGH MOLECULAR WEIGHT GLYCOPROTEIN EXPRESSED BY AN OVARIAN CARCINOMA CELL LINE, OVCA 433, IS RELATED TO GLUCOCORTICOID EFFECTS OF ALTERED CELL GROWTH, MORPHOLOGY, AND GROWTH PATTERN

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by Ellen Michele Weinstein

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ABSTRACT

INHIBITION OF CA 125, A NOVEL HIGH MOLECULAR WEIGHT GLYCOPROTEIN EXPRESSED BY AN OVARIAN CARCINOMA CELL LINE, OVCA 433, IS RELATED TO GLUCOCORTICOID EFFECTS OF ALTERED CELL GROWTH, MORPHOLOGY, AND GROWTH PATTERN

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OVCA 433 human ovarian carcinoma cells treated with $1x10^{-7}$M dexamethasone exhibit markedly decreased expression of CA 125, a nonmucinous, high-molecular weight (M_r=220,000) tumor-associated antigen as determined by an indirect immunoperoxidase technique with OC 125, a monoclonal antibody (MAb) recognizing the CA 125 determinant. Inhibition of CA 125 expression parallels dose-dependent glucocorticoid-induced effects leading to 32% growth inhibition and the appearance of cell morphological alterations, including enlargement, rounding, flattening, and multinucleation. CA 125 inhibition and altered cell growth appear to be events mediated through glucocorticoid receptors (GR) since other classes of steroid hormones, including aldosterone, estradiol, progesterone, and dihydrotestosterone fail to produce these effects. Furthermore, direct antagonism of CA 125 in vitro via functional blockade of cell surface bound glycoprotein with OC 125, anti-CA 125 MAb, produces 70% growth inhibition and profound alterations in cell growth pattern. OVCA 433 cells growing in OC 125-treated cultures become enlarged, markedly polygonal and assume neat, hexagonal arrays. Morphological alterations occur in a concentration-dependent manner at 3, 10, and 30 ug/ml with approximately 25%, 70%, and 100% of cells affected, respectively. These results suggest decreased cell surface CA 125 expression may mediate glucocorticoid-induced growth inhibition and morphological alterations, and suggest that the CA 125 determinant plays an important role in the control of ovarian carcinoma cell growth.

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INTRODUCTION

In 1975, Kohler and Milstein proposed a method for fusion of myeloma and murine spleen cells to create hybridomas capable of continous production of specific antibodies in vitro[1], termed monoclonal antibody (MAb). Murine monoclonal antibody technology has significantly impacted the direction of clinical diagnosis and management as well as introduced an unlimited supply of molecular and functional probes for study of cellular and molecular processes. One outgrowth of this innovation was the recognition that tumor cells and cells neoplastically transformed via viral or chemical induction, express cell surface molecular or macromolecular components which are often antigenically distinct from the normal cell surface repertoire. A number of murine monoclonal antibodies have been prepared against a variety of human neoplasms including colorectal, pancreatic, endometrial, ovarian, and breast carcinoma as well as melanoma and osteogenic sarcoma. The specificity provided by monoclonal antibodies for distinguishing neoplastic from normal tissue promises to revolutionize cancer diagnosis and therapeutics; monoclonal antibodies such as CA 125 have, in many cases, already become useful adjuncts to clinical diagnosis and management.

CA 125, an ovarian carcinoma tumor-associated antigen,

is a high molecular weight glycoprotein (M_r=220,000) expressed by 80% of nonmucinous epithelial ovarian carcinoma cells.[2,3] OC 125, a murine monoclonal IgG1 bearing a recognition site for the CA 125 epitope, was raised against OVCA 433 cells, a human serous cystadenocarcinoma cell line.[2] OC125 recognizes a distinct determinant shared by malignant ovarian carcinoma cells and fetal coelomic epithelium and is not found in detectable amounts in either adult or fetal ovary.[4] On this basis, CA 125 represents an oncofetal protein which may play a role in the complex processes regulating cell proliferation and differentiation.

Ovarian carcinoma is the second most common malignancy of the female reproductive system with over 18,500 newly diagnosed cases each year.[7] The disease often presents among women entering the sixth and seventh decades of life and, statistically, 1 in 70 females females will be affected. Despite new insights and technologic advances in the delivery of combined modality therapy, including surgery, chemotherapy, and radiotherapy,[8] the incidence and mortality rates of ovarian carcinoma have remained relatively constant over the past twenty years at 14 and 9 per 100,000 per year, respectively. Tragically, the disease is often asymptomatic in its early stages and over 2/3 of cases are diagnosed when disease is extensive and most refractory to treatment. Cytotoxic chemotherapy produces regression of residual tumor after cytoreductive surgery;

however, surveillance is difficult and even when a complete clinical response can be documented pathologically at a "second-look" laparotomy, tumor can recur in as many as 28% of cases.[9]

CA 125 can be detected on ovarian carcinoma cells and is elevated in the serum of 80% of ovarian carcinoma patients at the time of primary presentation.[2,10] A radioimmunometric assay developed by Bast et. al. for measurement of circulating CA 125 has made this tumor marker a useful adjunct to diagnosis and surveillance of ovarian carcinoma patients. Over 80% of ovarian adenocarcinoma patients have circulating CA 125 levels >35 U/ml at initial diagnosis, while <1% of healthy individuals express high levels of CA 125. In one retrospective study, elevated CA 125 could be detected as early as one year prior to clinical presentation of the disease.[11] While elevated levels of CA 125 occurring with a variety of benign conditions including menstruation, pregnancy, endometriosis, and pelvic inflammatory disease,[12,13,14] preclude widespread screening CA 125 levels to promote early detection, the serum CA 125 assay greatly aids in clinical decision making during therapy. Elevated CA 125 correlates with disease course in 80-95% of cases with normalization coinciding with disease regression post chemotherapy and rising titers coinciding with recurrence confirmed at "second look" procedures.[15] The CA 125 marker can also be detected at the

tumor cell surface in surgical specimens by immunohistochemical techniques. Koelma et. al. have found marker expression to be a useful adjunct to histology in pathological diagnosis.[16] In addition, several groups are currently investigating the utility of radionuclide-monoclonal antibody conjugates for diagnostic immunoscintigraphy and potential use for monoclonal antibody guided radioimmunotherapy.

Although the literature regarding the clinical utility of CA 125 continues to expand, the biological significance of the glycoprotein bearing the CA 125 epitope remains obscure. Recently, Karlan et. al. showed that the CA 125 determinant is highly sensitive to physiologic concentrations of glucocorticoids in vitro.[17] They have demonstrated that treatment of OVCA 433 cells with $1x10^{-7}$M dexamethasone results in a >95% inhibition of CA 125 release into culture media. Preliminary studies by immunofluorescence suggest a concordant decrease in cell surface CA 125 glycoprotein expression as well. Glucocorticoid-specific inhibition of CA 125 is temporally related to previously reported glucocorticoid inhibition of OVCA 433 cell growth, inhibition of urokinase and tissue plasminogen activator secretion, and profound morphological alterations.[18] In addition, Ishiwata et. al. have reported that treatment of several human ovarian carcinoma cell lines with dibutyryl-cyclic adenosine monophosphate (cAMP)

increases CA 125 release.[19] Cyclic AMP has been well-characterized as a common second messenger for a variety of cell surface hormone and growth factor receptors. Decreased CA 125 expression in the presence of hormones having a growth inhibitory effect and increased CA 125 expression in the presence of a potent growth signal transducer suggests a strong relationship between CA 125 expression and regulation of cell growth and function.

This thesis contributes to further definition of the hormone sensitive properties of CA 125 expression and its functional relationship to cell growth in vitro. In addition to examining CA 125 hormonal regulation, I investigated whether inhibition of CA 125 might play a role in the growth inhibitory effects of glucocorticoids. Studies employing MAb directed against the CA 125 epitope as a functional probe were initiated to assess specific effects of direct antagonism of this glycoprotein. If CA 125 represents an oncodevelopmental protein essential to the regulation of cell growth, effects on cell growth kinetics, morphology, and growth pattern might be expected with CA 125 antagonism in vitro by functional blockade of antigen with the anti-CA 125 Mab, OC 125.

Specific parameters investigated included:

1. Characterization of glucocorticoid effects on cell surface CA125 expression.

2. Characterization of the relationship between morphology and CA125 expression in glucocorticoid treated OVCA 433 cells.

3. Immunocytochemical localization of the CA125 determinant in glucocorticoid-treated and -untreated OVCA 433 cell using an indirect avidin-biotin complex immunoperoxidase technique.

4. Use of OC125 as a probe to assess effects of CA125 antagonism on OVCA 433 cell proliferation in vitro.

5. Characterization of the effect of CA125 antagonism with OC125 on OVCA 433 cell morphology and growth patterns.

LITERATURE REVIEW

I. GLUCOCORTICOID-INDUCED GROWTH INHIBITION

Glucocorticoids Alter Cell Growth

The anti-proliferative properties of glucocorticoids are well-documented.[20,21,22] Although several investigators have reported conflicting results of both stimulatory and inhibitory effects of glucocorticoids using a variety of cell lines, Durant et. al. point out that glucocorticoid effects on growth may greatly vary according to the choice of experimental model, the species, and the tissue being studied.[23] In addition, Rosner and Cristofalo observed differential response between transformed and untransformed cells; hydrocortsione consistently stimulated the growth of normal fetal lung fibroblasts while virus-transformed cells were growth inhibited.[24] Glucocorticoids are known to exert specific induction of key enzymes without affecting mean protein content; therefore, growth inhibition is not likely to represent nonspecific suppression of metabolic events. Consequently, glucocorticoid-induced effects on neoplastic cell growth offer a valid experimental model through which to study cell regulatory processes *in vitro*.

Primary Events Regulate Cell Growth

In order to engage in active growth, cells in resting phase must traverse a G_0-G_1 phase block or

restriction point. This cycling between growth arrested and proliferative stages is of some consequence since populations of cells are obligated both to reproduction and to preservation of terminal differentiation events, thus requiring individual cells to be highly dependent upon internal growth regulatory signals. Pardee et. al. recently reviewed the cell biology of normal and aberrant proliferation.[25] He reports that the most critical event in growth regulatory control occurs 2 hours prior to entry into S phase. Cells appear to be committed to initiate DNA replication at this restriction point, after release from which cells are capable of replication and division even in the absence of exogenous growth factors such as PDGF, EGF, and prostaglandins required in early G_1. Proliferation is thus controlled by events unique to G_1 phase rather than S, G2, or M. The latter phases are necessary for the completion of cell division amd terminal events (ie. cytokinesis) but do not depend upon molecular regulatory processes in the same way as G_0 and G_1.

Malignant Cells Exhibit Aberrant Response to Normal Growth Regulatory Processes

Neoplastic transformation may be seen as a defective regulaton of cell growth and differentiation induced by aberrant regulatin of the biological processes upon which cell growth depends. Croy and Pardee have characterized a

68,000 dalton protein, p68, produced in large amounts by transformed BALB/c-3T3 cells, which is a potential candidate for release of normal cells from the putative G_1 phase restriction point.[26] These authors theorize that this protein is constitutively expressed or stabilized in neoplastically transformed cells leading to escape of cells from normal growth control. They suggest that transient elevation of p68 represents a final commitment to induction of cell division requiring a complex sequence of early G_1 growth factor regulated events in order to occur.

Glucocorticoids Induce Growth Arrest in Neoplastic Cells at Defined Restriction Points

Glucocorticoids have been shown to induce a G_1 phase block in melanoma cells[27] as well as in several transformed cell lines including HeLa cells,[28] NHIK 3025,[29] and DDT_1 MF-2.[30] The G1 block requires the presence of glucocorticoids during the early or late G1 phases. Fanger et. al. have suggested a second steroid-dependent restriction point also exists in the beginning of S phase which extends the G2/M phase of the cell cycle in synchronized HeLa S_3 cells.[31] These studies show definitively that glucocorticoids can regulate progression through the cell cycle; however, knowledge regarding the mechanism of steroid-induced growth inhibition is presently limited.

Glucocorticoids May Regulate Cellular Response to Autocrine Growth Factors

Using the DDT_1 MF-2 cell line, a ductus deferens smooth muscle tumor cell line, which is sensitive to growth stimulation by androgens via androgen receptors (AR) while inhibited by glucocorticoids via glucocorticoid receptors (GR),[32] Norris et. al. found that glucocorticoids inhibit stimulation of cell proliferation mediated by AR enhancement. Inhibition occurs by induction of a G_0/G_1 block 18 hours after addition of glucocorticoids.[33] Interestingly, addition of exogenous PDGF to cultures overcomes this G_1 block independent of androgen receptor level. The authors interpreted independence of the glucocorticoid effect from AR as evidence for glucocorticoid modulation of autocrine rather than genomic events.

Subsequently, Syms et. al. reported that DDT_1 MF-2 cells produce c-sis proto-oncogene mRNA, which encodes a PDGF-like growth factor which is significantly depressed in non-proliferating cells.[34] PDGF is known to induce cell transition from resting to G_1 phase rendering them competent to respond to other growth factors. Glucocorticoids dramatically inhibit c-sis production in DDT_1 MF-2; however PDGF-like product continues to be significantly expressed in a glucocorticoid- resistant cell line, DDT_1 MF-2-GR. Norris et. al. suggested a mechanism for glucocorticoid modulation of oncogene expression by

demonstration that triamcinolone acetonide treatment of DDT_1 MF-2 cells prevents release of c-sis poly A+ transcripts into the cytoplasm and subsequent production of PDGF-like molecule.[35] This finding strongly supports the theory that glucocorticoid effects occur through modulation of cell growth factors or growth factor receptor expression.

Glucocorticoids have been shown to interfere with the action of several growth factors but effects vary according to primary stimulus. Studies performed on confluent, serum-starved 3T3 fibroblasts have shown that glucocorticoids, which usually do not affect proliferation under these conditions, enhance the stimulatory effect of fibroblast growth factor (FGF). In contrast, glucocorticoids significantly inhibit stimulation of DNA synthesis by exogenous epithelial growth factor (EGF) and prostaglandin $F2_a$.[36,37,38] Baker et. al. found that dexamethasone-induced enhancement of EGF stimulation in human foreskin fibroblasts parallels an increase in the number of membrane EGF receptors.[39] Fanger et. al. have similarly demonstrated membrane surface receptor modulation; EGF receptors increase following a 24 hour incubation of HeLa S_3 cells with 10^{-7} M dexamethasone.[40]

Alternatively, steroids may act through control of growth factor synthesis. Glucocorticoids have been shown to significantly inhibit cellular release of cytokines controlling differentiation and activation of lymphocytes

including interleukin-1 (IL-1) and colony stimulating factor.[41,42] Furthermore, Besedovsky et. al. have shown that when subpyrogenic doses of homogenous human-monocyte derived IL-1 or a recombinant form of IL-1 are administered to mice and rats, an increase in serum levels of ACTH and endogenous glucocorticoids ensues.[43] This effect suggests that endogenous glucocorticoids regulate IL-1 release via a feedback inhibitory circuit affecting cytokine production and activation, providing a physiologic correlation for corticosteroid pharmacological suppression of immune and inflammatory processes.

Glucocorticoids have numerous specific effects on biological and physiologic processes; therefore, multiple autocrine as well as non-autocrine growth regulatory interactions are likely to contribute to glucocorticoid growth inhibitory effects. Glucocorticoids are known to depress glucose uptake and impair ATP formation thereby decreasing the cellular energy supply available for maintenance of DNA, RNA and protein synthesis.[23] Glucocorticoids are also well known to depress synthesis of cholesterol and mevalonate derivatives, whereas mitogen-induced DNA synthesis and cell proliferation in lymphocytes is preceded by a peak of cholesterol synthesis. When cholesterol synthesis is directly inhibited by hydroxysterols, marked depression of blast transformation can be observed. Finally, polyamines, which play an

essential role in proliferation as well as development of mammalian cells, appear to be under glucocorticoid regulation.[44] Depletion of polyamines such as spermidine and spermine by inhibitors leads to suppression of cell proliferation in both normal and neoplastic cell cultures. In S49 mouse lymphoma cells, dexamethasone-induced growth inhibition correlated with decreased activity of ornithine decarboxylase, a key enzyme responsible for polyamine synthesis.[23] Glucocorticoids are also considered to be potent inhibitors of prostaglandin synthesis via inhibition of phospholipase A2 activity and resultant decreased availability of the fatty acid precursor arachidonic acid.[45]

Glucocorticoids exert profound affects on a variety of vertebrate tissue during embryonic development. These effects include induction of embryonic liver glycogen synthesis, maturation of intestine and retinal photoreceptor cells, as well as lung cytodifferentiation and accelerated synthesis of surfactant by Type II alveolar epithelial cells.[46] Often these effects are mediated by glucocorticoid induction of enzymes and proteins or augmentation of cellular sensitivity to endogenous signals such as enhanced pancreatic secretion of insulin in response to glucose. In fact, embryonic GR expression occurs relatively early during development, even before glucocorticoid-mediated events can be induced. The common denominator of steroid

effects, according to Ballard, is an acceleration of programmed events, ie. glucocorticoids serve as trigger affecting the timing and rate but not the sequence of developmental processes. It therefore seems likely that glucocorticoids inhibit control of cell proliferation and differentiation through targeted events which through further investigation may assist in definition of the molecular and biological processes governing development.

II. TUMOR-ASSOCIATED ANTIGENS

Many Tumor-Associated Antigens are Carbohydrate Stuctures

Cells transformed *in vitro* by viral or chemical tumor promoters or derived from tumor extracts have been recognized to express different profiles of cell surface structures from their nontransformed progenitor cells which are primarily carbohydrate in nature.[47] Monoclonal antibodies, directed against these determinants or tumor-associated antigens, recognize primarily carbohydrate structures on the extracellular domains of cell surface glycolipids and glycoproteins. Several groups have characterized monoclonal antibodies which bind determinants on the surface of ovarian carcinoma cells. Of 21 structurally defined epitopes found specifically in association with human epithelial ovarian carcinomas, 15 consist of a glycoprotein determinant, 6 consist of a protein determinant, and 2 consist of a determinant expressed both on low-molecular-weight glycolipid and glycoproteins.[9] [Appendix 1]

In general, carbohydrate changes in glycoproteins occur on three orders; a) increases in the number of asparagine-linked oligosaccharides resulting in higher molecular weight complexes than expressed by untransformed cells; b)increases in O-glycosylated mucin-type structures; and c)increases in peripheral side chains in N- or O-glycosidic linked structures due to either incomplete

synthesis or neosynthesis.[45]

Carbohydrate Synthesis May Be Aberrant in Neoplastic Cells

Many glycoproteins, such as hormones, mucins, immunoglobulins, and blood group determinants, are secretory proteins rendered soluble by their carbohydrate content which may vary from 2-90% by weight. Most are composed of short oligosaccharidic chains joined to a polypeptide core by an O-glycosidic linkage involving threonine and serine linkages. Alternatively, carbohydrate may be linked by an N-glycosidic linkage via an asparagine residue, in which case the oligosaccharide moiety is assembled on a dolichol carrier before transfer to the protein backbone. The protein moiety is assembled on a ribosome attached to the rough endoplasmic reticulum (RER) before passage through RER cisternae to the Golgi apparatus where glycosylation occurs. Monosaccharide units are transferred from UDP-sugars to growing polysaccharide chains in reactions catalyzed by specific glycosyltransferases. The mechanism behind synthesis of tumor-associated glycoprotein may involve defective transcription, translation, post-translational modification or enzyme specificity, any of which might lead to aberrant glycosylation.

CA 125 is a determinant carried on a high-molecular-weight glycoprotein (M_r=220,000) expressed on the surface of nonmucinous human ovarian carcinoma cells[48] CA

125 was initially defined by a murine monoclonal IgG_1 (OC 125) raised by immunization of BALB/c mice with OVCA 433 cells, a human serous cystadenocarcinoma cell line.[2,3] OC 125 recognizes distinct molecule expressed by OVCA 433 cells. During initial screening, its specificity for a carcinoma-associated determinant was established by virtue of its failure to recognize determinants expressed by nonmalignant cells or by an Epstein-Barr virus-transformed autologous B cell line established from lymphocytes from the donor of the OVCA 433 cell line progenitor. OVCA 433 cells growing in vitro express CA 125 on a cell surface glycoprotein as well as release significant quantities of an antigenically-identical epitope into the culture media. Circulating CA 125 can also be isolated from the sera of ovarian carcinoma patients and has been shown to be immunologically and physically similar to antigen isolated from the OVCA 433 cell line. Gel electrophoreisis and molecular size exclusion chromatography define the smallest molecular subunit expressing the epitope to be >200 kilodaltons; however, antigen released by OVCA 433 cells or isolated from human sera can be associated with a complex up to 10^6 daltons.[48]

The definitive nature of the CA 125 epitope, ie. whether the OC 125 binding site recognizes a protein or carbohydrate moiety of a high molecular weight glycoprotein, remains controversial. Hanisch et. al.

originally characterized CA 125 as a carbohydrate structure sensitive to periodate oxidation and cleavage by N-acetylneuraminic acid.[49] Davis et. al. disputed this result based on their studies showing relative stability of CA 125 subjected to periodic acid treatment, and suggested that Hanisch's contradictory results were obtained under conditions which destroyed the integrity of CA 125 by virtue of its heat sensitivity. This group further established complete loss of CA 125 activity after protease digestion and urea treatment and demonstrated retention of activity despite exhaustive treatment with exoglycosidases.[48] These findings led Davis et. al. to conclude that CA 125 represents a conformationally-dependent epitope with recognition of a protein perhaps in association with carbohydrate dependent upon preservation of the integrity of secondary and tertiary structure. Recently, Leoni et. al. reported two monoclonal antibodies, CAMOv2 and CAMOv8, raised against ovarian carcinoma cyst fluid glycoproteins, which can detect saccharidic chains expressed on the same molecule and occasionally coexpressed with CA 125.[50] These authors are in agreements with Davis' hypothesis of a conformational epitope suggesting that two saccharidic side chains, recognized by CAMOv2-CAMOv8 and a protein backbone contribute to the structure complementary to the OC 125 F_{ab} site.

Immunopathological Correlations of CA 125 Expression

Current definitions of cancer stress that processes leading to neoplastic transformation may reflect blocks or aberrations in the regulatory processes controling normal growth and differentiation.[25] In support of this theory, Hakamori points out that most tumor-associated antigens are defined by monoclonal antibodies recognizing normal cell surface molecules, usually absent or present in insignificant quantities, which have been augmented or modified by neoplastic transformation in a way which renders them more antigenic.[51] For example, several TAA's appear to represent derivatives of blood group antigens normally not expressed in an individual, ie. A-like antigen may be present in colonic cancers in hosts of O or B type. Often, determinants are not restricted to neoplastic expression, as certain antigens may be detected in a tissue-specific manner or at specific points during normal embryonic development, earning their classification as oncofetal proteins.

CA 125 represents one such glycoprotein demonstrating oncofetal expression. CA 125 is present in normal adult tissue derived from coelomic epithelium and nonmucinous common epithelial ovarian tumors; however it cannot be detected in normal fetal or adult ovary.[6] This conforms with the common belief that the majority of ovarian tumors are felt to arise from the germinal epithelium of coelomic

origin which covers the ovarian surface and lines subcapsular cysts. Immunohistochemical localization of fetal CA 125 detects antigen expression in embryonic pleura, pericardium, peritoneum, mullerian duct, and amnion,[6] as well as in normal adult tracheobronchial lining.[52] CA 125 is also present in a variety of human secretions including human milk,[50] cervical mucus,[53] and seminal fluid.[48] In terms of its neoplastic expression, Kabawat has demonstrated CA 125 in 82% of serous cystadenocarcinomas and 100% of benign and borderline ovarian tumors. A variety of nonovarian malignancies express CA 125 as well including adenocarcinomas of the pancreas,[50] endometrium, fallopian tube, and endocervix[54] as well as a small minority of lung, breast, colorectal carcinomas and melanomas.[9]

Feizi has suggested carbohydrate structures associated with neoplastic and transformed cells represent developmental and growth regulatory proteins.[55] He invokes as proof the existence of highly-conserved oligosaccharidic structures such as the fucosylated N-acetylactosamine bound by Mab anti-SSEA-1, an epitope expressed by the 8-cell-stage mouse embryo and shared with gastrointestinal glycoproteins, human ovarian cyst proteins, human adenocarcinoma glycolipids, and human milk oligosaccharides. In addition, if one takes note that one of the first reports of a tumor-associated antigen demonstrated that the anti-melanoma monoclonal antibody which defined the TAA significantly

suppressed human melanoma tumor growth in athymic mice,[56] the concept of tumor-associated antigen expression as a requirement for neoplastic growth is a highly provocative one.

The observation that activated oncogenes including erbB, fms, neu, sis, and ras encode products expressed as trans-membrane glycoproteins with large extracellular domains [57,58,59] lends further support to this hypothesis. Recently, Drebin et. al. demonstrated MAb anti-p185, when used to antagonize neu-oncogene encoded product, p185, expressed in high levels by neuroblastomas and various adenocarcinomas, significantly inhibits anchorage independent growth of neu-transformed NIH 3T3 cells[60] as well as tumorigenic growth of transformed cells implanted into nude mice.[61]

The characterization of another growth-related melanoma-associated antigen, p97, recognized by MAb 9.2.27, represents one of the most detailed physical and functional analyses to date of a glycoprotein implicated in growth regulation. Melanoma-associated p97 is a sialylated transmembrane carbohydrate glycoprotein (M_r=250,000) expressed by melanoma, nevi, and fetal intestine.[62] In a study which precedented the use of monoclonal antibodies as functional probes for growth and differentiation effects, Harper and Reisfeld demonstrated that anti-p97 MAb inhibits anchorage-independent human melanoma cell growth and blocked

cell spreading and adhesion to substratum.[63] In 1986, Rose et. al. purified, cloned, and sequenced p97 mRNA in order to determine its amino acid sequence and core carbohydrate structure.[64] Interestingly, the epitope recognized by Mab 9.2.27 exhibited 39% homology to human serum transferrin in the region of the iron-binding pocket. Although the growth regulatory properties of transferrin have been well-established, no experimental proof exists demonstrating p97 uptake of transferrin-bound iron; therefore, a growth regulatory role for p97 remains speculative until such functional studies can be performed.[65]

A new approach to the characterization of oncofetal antigens which has recently appeared in the literature is the direct analysis of proliferation-associated determinants. Devendra et. al. raised MAb anti-Proliferation Associated Antigen (anti-PAA) by immunization of mice against activated human T-cells in mixed-lymphocyte cultures(MLC).[66] These authors found a 2-6 fold increase over resting T cells of PAA expressed by MLC- and phytohemagglutinin-stimulated T cells. Transformed hematopoeitic cell lines exhibited dramatic increases in PAA with an acute lymphocytic leukemia T cell line and an EBV-transformed B cell line showing 15- and 16-fold increased anti-PAA binding, respectively. Anti-PAA demonstrated an oncofetal pattern of staining with significant quantities of antigen expressed by chorionic villi, trophoblasts, actively

proliferating normal adult skin, and colonic epithelia, as well as benign hyperplastic tissue. As more and more investigators undertake projects aiming toward structural and functional definition of tumor-associated antigens, their biological relevance ought to lead to new concepts of how extracellular and membrane structures contribute to the appearance of neoplastic clones and tumorigenic growth.

MATERIALS AND METHODS

CELL LINES OVCA 433 human ovarian carcinoma cells, derived from a human serous cystadenocarcinoma of the ovary as described previously,[2,67] were grown at 37°C, 5% CO_2 in Minimal Essential Media (MEM)(Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (FCS), 1mM sodium pyruvate, 2mM L-glutamine, and 100 U/ml penicillin/streptomycin (Gibco). SK-OV-3 and Caov-3 cell lines, derived from human ovarian adenocarcinomas, were grown in McCoy's M5A and Dulbecco's Modified Eagle's Media (Gibco), respectively, with 10% FCS, 2 mM L-glutamine, and 100 U/ml penicillin/ streptomycin added.

BIOLOGICALS Dexamethasone (Steraloids, Wilton, NH) was prepared freshly in deionized water at 1 X 10^{-4}M, sterilized through Millex-GS 0.22 um filters (Millipore, Bedford, MA) and diluted appropriately before addition at 1:2 to 1:10 to final concentration of 10^{-6}M-10^{-10}M Dexamethasone, cortisol, aldosterone, estradiol, dihydrotestosterone, and progesterone used in the specificity experiments was freshly prepared in culture media from stocks stored in 100% ethanol at -20°C.

OC 125 MAb, purified from mouse ascites, kindly supplied by Dr. Vincent R. Zurawski, Jr.(Centocor, Malvern, PA) was diluted in fresh media from stocks stored at -80°C in Tris:EDTA buffer. Before addition to cell culture, OC

125 was filter sterilized through Millex-GV low protein binding 0.22um millipore filters (Millex, Bedford, MA). Whole mouse IgG and mouse IgG_1 F_c fragment (Jackson Imuunoresearch, West Grove, PA) and mouse IgG_1 Kappa from MOPC 21 ascites (Sigma, St. Louis, MO) were similarly prepared.

IMMUNOCYTOCHEMISTRY An indirect ABC immunoperoxidase technique based on a method previously described by Childs et. al.[68] was employed. Briefly, OVCA 433 cells were harvested with 0.25% trypsin and replated in dual-chambered Lab-Tek Tissue Culture Chamber/Slides (Miles Scientific, Naperville, IL) at a seeding density of $8.5x10^4$ cells/chamber. After 4 days, confluent monolayers were washed with 0.01M Na Phosphate, .015M NaCl pH=7.4 (PBS) and fixed overnight at 4^oC in 4% EM-grade paraformaldehyde (Electron Microscopy Sciences, Fort Washington, PA) in PBS. Fixed monolayers were washed and incubated with 6% normal goat serum to block nonspecific staining. Primary antibody, MAb OC 125, was applied at to monolayers at 1-40 ug/ml overnight at 4^oC. Cells were rewashed with PBS before application of biotinylated anti-mouse IgG (Vectastain ABC kit: Vector Laboratories, Burlingame, CA), 0.1% crystalline-grade bovine serum albumin (BSA) (Sigma, St. Louis, MO) in PBS for 30 minutes at RT. After several washes in PBS, horseradish peroxidase avidin-conjugate, prepared 15-30 minutes in advance, was applied to

monolayers for 30 minutes. Cells were again washed and a substrate prepared in the following manner applied for 7-10 minutes or until a blue-black pigment no longer continued to form. To prepare substrate, 6 mg diaminobenzidine and 0.33 g nickel ammonium sulfate (Sigma, St. Louis, MO) were added to 30 ml of .05M Sodium Acetate buffer, pH=6.0, sonicated, filtered and 90 ul 30% H_2O_2 added immediately before use. The substrate reaction was quenched in tap water and coverslips applied over 15% glycerol.

PHOTOGRAPHY Photos demonstrating glucocorticoid- and OC 125-induced morphological alterations were taken under a Zeiss phase contrast microscope with 10X, 20X, and 40X objectives through a green interference filter. Photographs demonstating immunocytochemical localization of CA 125 were obtained with the aid of a Sony Differential Interference Computerized Videoplan image processor.

MICROCULTURE CELL GROWTH ASSAY Growth inhibitory effects of CA 125 and dexamethasone were assessed using a methylene blue uptake previously described by Finlay et. al.[69] OVCA 433 cells were harvested with 0.25% trypsin and replated at 2000 cells/well in 96-well flat bottomed-plates with varying concentrations of OC 125 or dexamethasone. Days 1-7 post seeding, culture media was removed and 200 ul of 0.5% methylene blue/50% ethanol was added to each well for 30 minutes. After solubilization of cells in N-lauroyl-sarcosine, plates were subsequently washed in deionized

water baths until rinsewater cleared . Methylene blue uptake in fixed monolayers was correlated with absorbance at 600 nm determined on a Titertek Multiskan Plus ELISA (enzyme-linked immunosorbent assay) plate reader.

CONDITIONED MEDIA Conditioned media enriched with CA 125 was obtained by growing OVCA 433 cells to confluence 3-4 days, changing the media, and then permitting CA 125 to accumulate over a 7 day period before harvesting.

RESULTS

Dexamethasone-induced morphological alterations

OVCA 433 ovarian carcinoma cells were harvested with trypsin and replated with fresh media or $1x10^{-7}M$ dexamethasone and permitted to grow to confluence. After 4 days in culture, dexamethasone-treated cells exhibited significantly altered morphology as compared to controls; cells became enlarged, flattened, and rounded with disappearace of cell processes as compared to controls. (FIG. 1) There was significant variation in dexamethasone-sensitivity among a cell population as within a cell monolayer multiple clusters of cells with altered morphology could be observed while many cells remained unaffected. The earliest observable effect was the appearance of large, multinucleated cells 48 hours after plating. (FIG. 1)

Immunocytochemical detection of CA 125

To assess the effect of dexamethasone on OVCA 433 CA 125 expression, OC 125-binding sites were determinied using an indirect immunoperoxidase technique. OVCA 433 minicultures were seeded with $8.5x10_4$ cells and fresh media or media containing $1 \times 10^{-7}M$ dexamethasone and grown to confluence (4d) before fixation and staining with OC 125. In this technique, a secondary antibody-peroxidase conjugate, anti-mouse IgG, when complexed to cell surface bound CA 125 undergoes a substate reaction depositing a characteristic

blue-black pigment *in situ*. Dexamethasone-treated OVCA 433 cell monolayers consistently stained less intensely than untreated controls. When concentrations ranging from 10^{-5}M to 10^{-9}M dexamethasone were tested, this effect occurred in a dose dependent manner with maximal CA 125 inhibition observed at 10^{-6}M and 10^{-7}M dexamethasone. To compare the effect of different classes of steroid hormones on inhibition of CA 125 expression, OVCA 433 human carcinoma cells were treated with $1x10^{-6}$M concentrations of estradiol, dihydrotestosterone, progesterone, aldosterone, cortisol, dexamethasone or no steroid at all. CA 125 inhibition was found to be specific to the physiologic and synthetic glucocorticoids, cortisol and dexamethasone, while other classes of steroids yeilded no effect.

As observed with dexamethasone-induced morphological alterations, there was marked heterogeneity of staining intensity among individual cells or islands of cells within glucocorticoid-treated monolayers. In general, smaller cells and those with cytoplasmic extensions growing in a vermiform pattern present in dexamethasone-treated and-untreated monolayers stained most intensely. These cells exhibited cell surface and diffuse granular cytoplasmic staining; whereas the enlarged, flattened, polygonal cells more characteristic of the cortisol- and dexamethasone-treated monolayers demonstrated attenuated or a faint blush of staining often in a perinuclear distribution.(FIG. 2) No

correlation was observed between the presence of multiple nuclei and staining intensity. Cell monolayers in which the primary antibody incubation step with OC 125 was ommitted demonstrated only minimal background staining.

In order to define whether a quantitative relationship exists between stereotypic dexamethasone-induced morphology and CA 125 expression, I attempted to analyze immunoperoxidase-stained steroid-treated and -untreated OVCA 433 cells using an image processor with gross morphometric and gray scale analysis capabilities. Unfortunately, this line of investigation could not be pursued secondary to poor contrast and inaccessibility of equipment with potential for morphometric analysis of microscopic specimens.

Immunolocalization of CA 125 Determinant

To further delineate differences in the pattern of staining induced by dexamethasone, immunoperoxidase-stained, fixed monolayers of OVCA 433 cells were observed with the aid of a video-enhanced image processor and differential-interference screen to permit localization of intracellular CA 125. The majority of cells in the control population exhibited dense immunoprecipitate at the cell surface. (FIG. 3) Cells treated with 10^{-7}M dexamethasone, in contrast, frequently exhibited decreased cell surface staining relative to controls and a dense, granular sphere of immunoprecipitate restricted to the perinuclear area. (FIG. 4) The population of cells displaying this pattern of

staining was entirely consistent with the glucocorticoid-sensitive subpopulation displaying increased cytoplasmic volume and polygonal growth. At this level of resolution, staining could not be definitively localized to the perinuclear cytoplasm or the nuclear membrane.

Dexamethasone effect on cell growth

To determine the effect of glucocorticoids on cell growth rate, OVCA 433 ovarian carcinoma cells, grown in 96-well plates in the presence of varying concentrations of dexamethasone were assayed using the microculture cell growth assay. Dexamethasone inhibited growth up to 32% over controls occurred in a dose-dependent manner with maximal inhibition occurring at 10^{-6}M and 10^{-7}M dexamethasone.(FIG. 5, FIG. 6) Growth inhibition was most noticeable day 4 and 5 post seeding although treated and untreated cultures ultimately grew to similar density by day 7.

Antagonism of CA 125 determinant

Because the results above suggest that GR mediated alteration of cell morphology is associated with decreased expression of CA 125, I examined this relationship further by direct antagonism of CA 125 determinant via induction of a functional blockade with OC 125, anti-CA 125 MAb. Interestingly, MAb antibody OC 125, added to freshly plated OVCA 433 cells *in vitro*, induced profound alterations in morphology and growth pattern at 4-5 days. Cells became

markedly enlarged and polygonal with centralization of nuclei, cytoplasmic thinning, and decreased granularity.(FIG. 7) These changes occurred in a dose dependent manner at 3, 10, and 30 ug/ml with approximately 25%, 70%, and 100% of cells affected, respectively. In contrast to normal OVCA 433 growth which is primarily disordered and dependent upon contact-inhibition, OC 125-treated cells assumed a neat, hexagonal array with few cells overlapping. While untreated cultures reached confluence at 4-5 days, OC 125-treated cultures were considerably less dense and just beginning to extend cytoplasmic bridges between islands of cells at 4 days, characteristic of preconfluent growth. Within 24-48 hours of the appearance of peak morphological alterations, OC 125-treated cells consistently lifted off the plastic in sheets of cells while control OVCA 433 cells remained firmly adherent throughout the growth period. Cell detachment in wells plated with 10 ug/ml OC 125 occurred >24 hours earlier than detachment in wells with 30 ug/ml OC 125.

Specificity of the OC 125 effect

In order to demonstrate that recognition of CA 125 determinant as opposed to nonspecific effects of immunoglobulin or mouse ascites is a requirement for growth effects, OVCA 433 cells were incubated with 10 ug/ml whole mouse IgG, mouse IgG F_c fragment, or a nonspecific monoclonal immunoglobulin raised in MOPC 21 mouse ascites.

None of these antibody preparations altered morphology or growth pattern.(FIG 8) Similarly, cells incubated with MAb directed against irrelevant epitopes [ie. anti-tissue plasminogen activator and anti-HCG (beta subunit] were morphologically identical to control OVCA 433 cells at confluence.(FIG. 9) OC 125 at 10 ug/ml also failed to affect morphology, growth pattern, and adherence properties of Caov-3 and SK-OV-3 cell lines, two ovarian carcinoma cell lines previously shown to express no or insignificant quantities of CA 125.[70] (FIG. 10).

Finally, to confirm that the OC 125 growth effect is mediated via specific recognition of cell-bound determinant and not by antagonism of free CA 125 activity, OVCA 433 conditioned media known to contain high quantities of CA 125 was added to freshly plated cell cultures containing 30 ug/ml OC 125 plating. CA 125-enriched conditioned media failed to inhibit the OC 125 effect on cell growth and growth pattern, nor was inhibition of dexamethasone effects observed at 10^{-7}M steroid.

Determination of OC 125 effect on cell growth

When increasing concentrations of OC 125 were added to OVCA 433 cells *in vitro*, a dose-dependendent growth inhibitory effect of 72% at 30 ug/ml and 26% at 10 ug/ml was noted. (FIG. 11, 12) Growth inhibition was observed as early as 48 hours post seeding of cultures. Freshly seeded OVCA 433 cell cultures display rapid exponential growth

which declined with time in culture as cells approached confluence. Interestingly, this growth rate decline was accelerated in cultures containing 10 and 30 ug/ml OC 125 (FIG. 11) and cells entered stationary phase at 6-7 days as did controls despite markedly decreased culture density.

DISCUSSION

Ovarian carcinoma cells, OVCA 433, grown in culture media containing $1x10^{-7}M$ dexamethasone demonstrate 30% growth inhibition and significant morphological alterations. In agreement with previous findings,[18,71] a subpopulation of dexamethasone-sensitive cells become enlarged, loosely packed, flattened, and more polygonal. Using an immunoperoxidase technique, this work demonstrates that the dexamethasone-sensitive subpopulation of OVCA 433 cells is identical to a subpopulation of cells exhibiting significant inhibition of CA 125 cell surface expression in the presence of $10^{-7}M$ dexamethasone. This modulation of cell surface CA 125 appears to be mediated by steroid-specific GR, previously demonstrated in these cells,[17] since CA 125 inhibition was observed only for OVCA 433 cells treated with dexamethasone or cortisol. Treatment with other steroids, including estradiol, dihydrotestosterone, aldosterone, and progesterone in equivalent concentrations failed to produce altered morphology or antigen expression in these cells. In addition, peak inhibition of CA 125 was observed at glucocorticoid concentrations corresponding with those necessary to produce maximal growth inhibition in the microculture growth assay, suggesting CA 125 expression and growth inhibition of intimately related processes.

In order to determine whether decreased cell surface

expression of CA 125 mediated by GR is the primary event leading to induction of growth inhibition, a functional blockade of CA 125 determinant was accomplished by incubating freshly plated OVCA 433 cells with varying concentrations of OC 125, anti-CA 125 MAb. OVCA 433 cell monolayers treated in this manner exhibited dramatic changes in morphology and cell growth at 3, 10, and 30 ug/ml OC 125 with 25%, 70% and 100% of the cells affected, respectively. Cells became significantly enlarged, flattened, and polygonal with decreased granularity and an increased cytoplasmic:nuclear ratio. Monolayers demonstrated complete loss of the "garlands and whorls" pattern of growth described by Ioachim et. al.[72] characteristic of ovarian cystadenocarcinoma cell lines; rather they assumed a nonoverlapping, hexagonal array and, in some areas, cells adjacent to bare plastic resembled the epithelial lining found in microcysts of human serous cystadenocarcinoma. (FIG. 13).

In addition, OC 125 significantly inhibits OVCA 433 cell growth by 30% and 70% at concentrations of 10 and 30 ug/ml, respectively. At these concentrations, OC 125 induces OVCA 433, which grow firmly adherent to plastic, to lift off the culture dish in sheets of cells as they approach stationary phase. OC 125 interference with some mechanism of cellular adherence to substratum is likely responsible for the data points obtained at day 6 and 7 in

the microculture growth assay (FIG. 10, 11) reflecting the failure of cells in these wells to remain attached to plastic during fixation in ethanol. Adequate fixation depends upon the availability of matrix proteins which normally contribute to cell adhesion, and therefore, these observations may be consistent with an OC 125-induced modulation of proteoglycans synthesis or secretion. Paradoxically, disassociation from the culture dish appeared earlier in cultures plated at 10 ug/ml than at 30 ug/ml. One explanation for this finding may be that the presence of OC 125 in vitro is a biological stressor inducing increased utilization of nutrients and growth factors available in the media, resulting in the arrest of nonessential metabolic activity. The increased metabolic state concomitant with active cell proliferation may also be considered as a biological stressor and may compound the OC 125 effect accelerating consumption of cell nutrients. This would explain why cell detachment occurs precociously in more actively proliferating OVCA 433 cultures containing 10 ug/ml OC 125 while severely growth inhibited cultures with 30 ug/ml are relatively protected. Accelerated nutrient consumption may also explain why OC 125-treated OVCA 433 cells enter stationary phase at decreased cell density relative to controls; however, the onset of stationary phase primarily may be a function of time in culture and the half-lives of competence factors rather than cell density. It is

also interesting to note that many cell matrix proteins such as fibronectin are also considered to be competence factors,[26] and, therefore, cell proliferation and two-dimensional configuration may be dependent on factors mediating cell adhesion.

In order to establish whether growth inhibition and altered morphology occur through specific interaction of MAb with the CA 125 determinant, OVCA 433 and two additional human ovarian carcinoma cell lines which do not express CA 125 were grown to confluence in the presence of OC 125. The cell enlargement and polygonal growth which was observed in OVCA 433 cells failed to occur in either Caov-3 or SK-OV-3 cells after a 4 day incubation period with OC 125. Littlefield previously documented that CA 125 is found in high quantities (>1000 U/ml) in the media of 7 day OVCA 433 cultures but is not dectable in either Caov-3 or SK-OV-3 cultures. In addition, OVCA 433 cells were grown in the presence of affinity-purified whole mouse IgG and mouse IgG F_C fragment, as well as several monoclonal immunoglobulins recognizing irrelevent epitopes, none of which produced stereotypic effects. These findings confirm that morphological and growth effects noted are specific to recognition of the CA 125 determinant. Failure of free CA 125 found in high quantities in OVCA 433 7d conditioned media to inhibit the OC 125 effect strongly suggests that a specific ligand interaction at the cell surface membrane is

a requirement for altered growth pattern since this effect cannot be overcome by effectively adding back CA 125 product to culture media.

These experiments suggest that active cell proliferation is in part dependent upon the availability of of a high-molecular weight glycoprotein bearing the CA 125 epitope on the cell surface membrane. Decreased cell surface availability of CA 125 mediated by glucocorticoids or functional blockade with OC 125 MAb is associated with growth inhibition and morphological alterations. Although the relationship between growth patterns observed with glucocorticoids and OC 125 is uncertain, they bear several similarities (ie. increased cytoplasmic volume and polygonal growth) which suggest that common biological events may mediate both. It is interesting to note that OVCA 433 cells harvested with trypsin from monolayers grown to confluence in the presence of OC 125 and replated into culture flasks containing fresh media exhibit poor plating efficiency with only a minority of cells adhering to plastic in colonies <40 cells at 4 days. That these colonies demonstrate active, albeit significantly depressed growth, suggests that decreased CA 125 expression represents a partially reversible commitment step necessary for cells to traverse the cell cycle or to enter into terminal differentiation. Alternatively, adherent cells growing in fresh media may represent an OC 125-resistant subpopulation of cells which

have to some extent escaped the inhibitory effects of Mab.

This work demonstrates significantly decreased cell surface expression of CA 125 determinant in cells treated with 10^{-7}M dexamethasone as compared to controls. Hakamori and Kannagi[73] have theorized that cell surface antigen recognition is highly sensitive to antigen concentration and crypticity, where crypticity refers to modification of antigen secondary or tertiary structure or the configuration of the glycoprotein in the membrane lipid bilayer. It is unlikely that the inhibition of antigenicity induced by glucocorticoids, well known to affect cholesterol synthesis and membrane fluidity, represents a dilution of cell surface antigen via migration since cell surface glycoproteins are relatively immobile structures regulated by regional cytoskeletal elements and substratum. Modification of the CA 125 determinant rendering it functionally obsolete via enzymatic processing is possible and its structural derivative could not be distinguished by immunoperoxidase staining.

Little is known regarding mechanisms behind the regulation of cell surface CA 125 expression, although the appearance of altered intracellular distribution of CA 125 in glucocorticoid-treated OVCA 433 cells may shed light on how cell surface antigen modulation occurs in actively proliferating cells. Immunoperoxidase-stained OVCA 433 cells at confluence exhibit a dense precipitate at the cell

surface, reflecting CA 125 membrane expression; staining in dexamethasone and cortisol-treated OVCA 433 cell monolayers at confluence appears to be primarily restricted to a perinuclear granular deposit. This perinuclear distribution of CA 125 may represent neosynthesis or neoglycosylation occuring in response to loss or modification of cell surface CA 125 or as a direct effect of glucocorticoids. Although carbohydrate hormone receptors have been documented in the nuclear membrane,[74] the spherical configuration and granularity of stain rules against nuclear membrane staining. This distribution, however, is consistent with the rough endoplasmic reticulum and Golgi apparatus where glycoprotein synthesis and glycosylation is known to take place.[75,76,77]

Rosen et. al. have suggested that the most likely precursor storage site for carbohydrate receptors is the Golgi apparatus and that glycosylation serves as the rate limiting step for receptor synthesis.[78] Receptor expression is accomplished by integration of Golgi vessicles containing glycosylated integral membrane proteins into the cell surface. Membrane turnover via shuttling of Golgi vessicles has been induced as another mechanism governing regulation of hormone receptors.[79] In fact, Palade has proposed a protective role for Golgi vessicles; he postulated that the Golgi apparatus serves as an intermediate in a cycling mechanism where receptor is internalized to escape

degradation (ie. lysosomal) and subsequently reincorporated into the surface membrane.[80] Increased perinuclear CA 125 in parallel with decreased cell surface expression may similarly reflect receptor internalization and storage within the Golgi vessicles as opposed to antigen loss followed by neosynthesis. Delineation of a primary mechanism, however, will require further investigation via classical pulse-chase S-methionine labeling in combination with electron microscopy.

These studies suggest that modulation of cell surface CA 125 expression is a process which may serve an integral function for control of cell proliferation and cell:cell interactions in ovarian carcinoma. One caveat presented by these results, however, is the heterogeneity observed for expression of CA 125 as well as for sensitivity to dexamethasone among OVCA 433 cells. Heterogeneous immunoperoxidase staining is also highly characteristic of primary human serous ovarian adenocarcinomas which display intimately mixed positive and negative cells when stained for CA 125.[3,4] Heterogeneous expression is not unique to the CA 125 marker. A host of tumor-associated antigens are variably expressed within primary and metastatic tumor specimens as well as within tumor cell lines.

Several theories have been invoked to explain antigen modulation including antigen shedding and accelerated frequency of mutational events. If one assumes that a tumor-

associated antigen is an aberrantly processed glycoprotein conserved because its expression conveys a selective advantage, one must also consider that variable expression may arise because an antigen plays a function which is not critical to preservation of neoplastic growth. This statement does not appear to hold true for CA 125, since 100% of cells can be induced to enter a growth-inhibited or arrested phase and undergo morphologic alterations in the presence of high doses of OC 125 despite variable antigen expression. Therefore, while excessive expression of an oncodevelopmental antigen, such as CA 125, normally suppressed in terminally differentiated tissue, is one possible mechanism leading to uncontrolled proliferation, antigen heterogeneity suggests that, although they may be clonally-derived, as tumors progress they become mosaics of subclones with various cell regulatory escape mechanisms. The biological processes leading to transformation are as complex as the vast number of molecular interactions which must take place for normal growth regulation to occur; functional definition of tumor antigens, however, will likely shed light on how tumorigenic cells escape constitutive growth regulation. In addition, perhaps definition of TAA function will inspire innovative approaches to pharmacological inhibition and permit more prudent selection of antigens for use as prognostic markers or targets for radionuclide-antibody conjugates.

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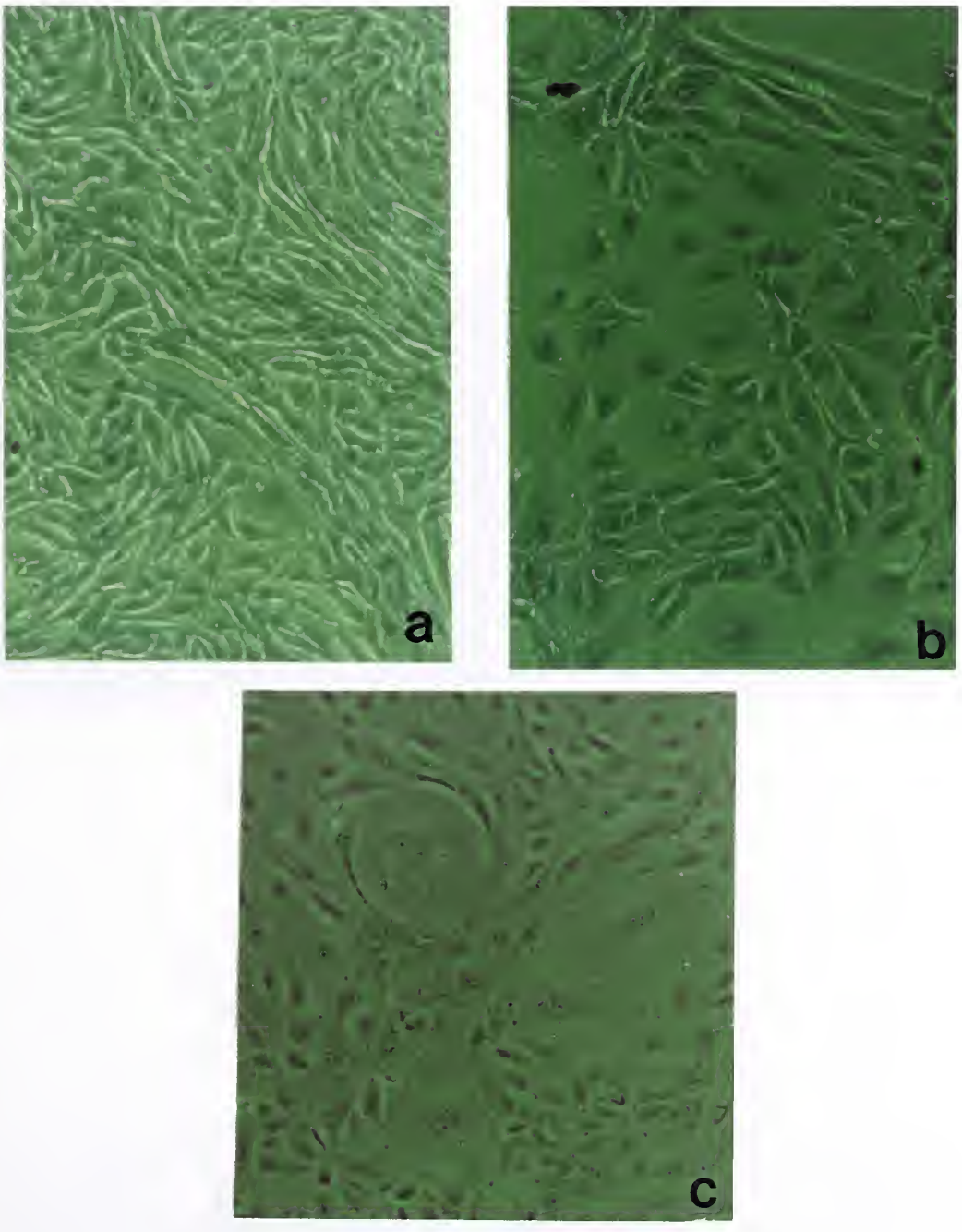

FIG. 1. Effect of dexamethasone treatment on OVCA 433 cell morphology. Freshly plated cell were grown in (a) MEM, 10% FCS or (b) MEM, 10% FCS + 1 X 10-7M dexamethasone for 4 days prior to photography. Note enlargement, flattening, and increased polygonal growth as compared to controls. (c) Appearance of large, multinucleated cells in dexamethasone-treated cultures 48 hours after plating.

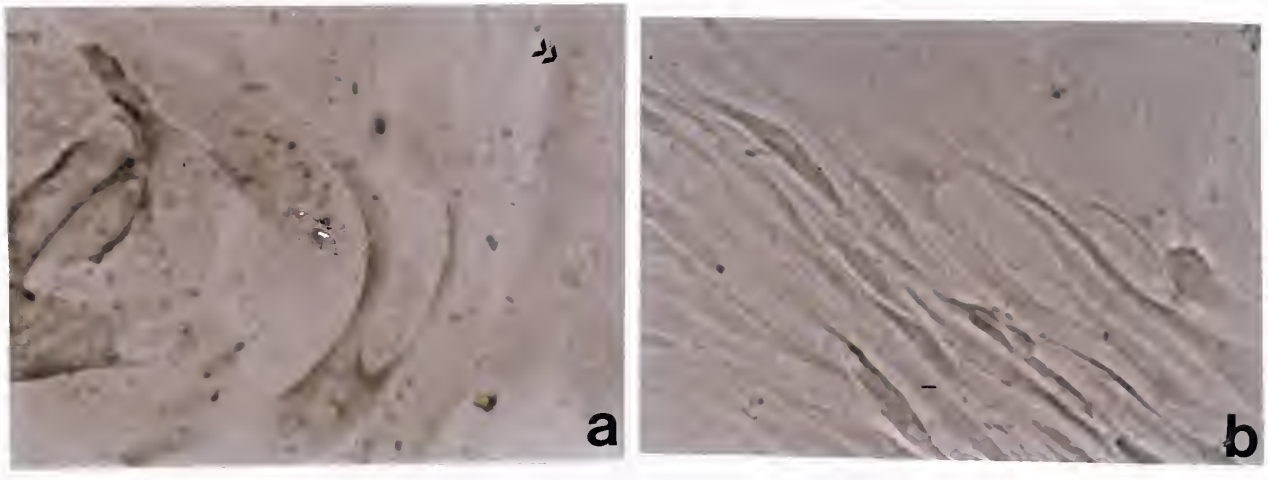

FIG. 2. Effect of dexamethasone treatment on OVCA 433 CA 125 expression. Immunohistochemical staining of (a) dexamethasone-treated and (b) -untreated OVCA 433 cells fixed X 24 hours in 4% paraformaldehyde demonstrates decreased expression of OC 125 cell surface binding sites by cells exhibiting dexamethasone-induced morphological alterations. (a) Note heterogeneity of staining within dexamethasone-treated monolayer. Smaller cells and cytoplasmic extensions stain most intensely while enlarged, polygonal cells (arrow) exhibit mostly diffuse, perinuclear staining.

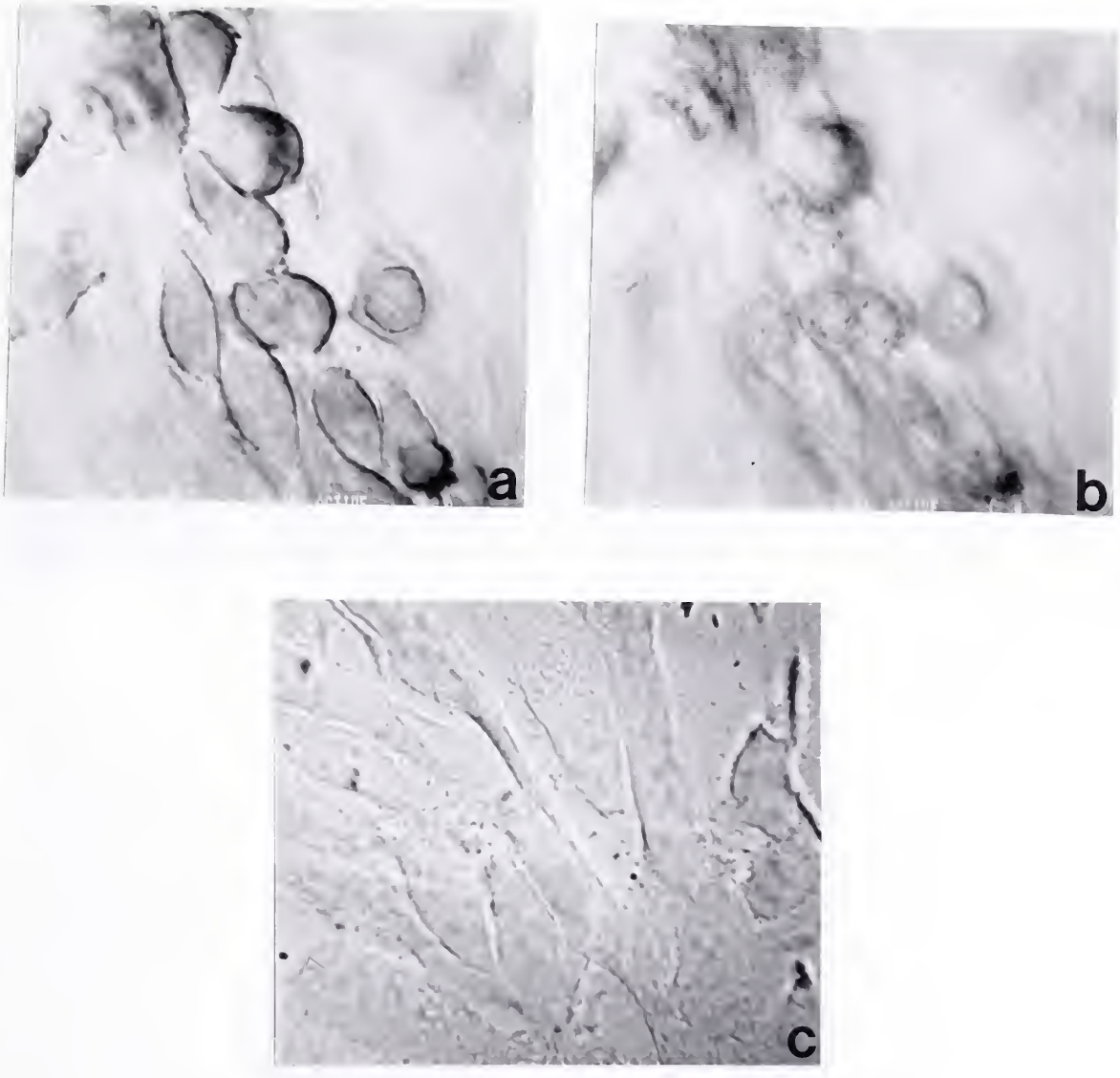

FIG. 3. Immunolocalization of CA 125 in OVCA 433 ovarian carcinoma cells. Cells stained with OC 125 using DAB/Ni ammonium sulfate substrate photographed with the aid of a video-enhanced differential interference camera system. (a) and (b) Two focal planes exhibited to demonstrate staining is predominantly restricted to cell surface membrane and cytoplasmic processes. (c) OVCA 433 cells stained as above except OC 125 primary incubation step ommited to demonstrate specificity of substrate reaction for OC 125 bound sites.

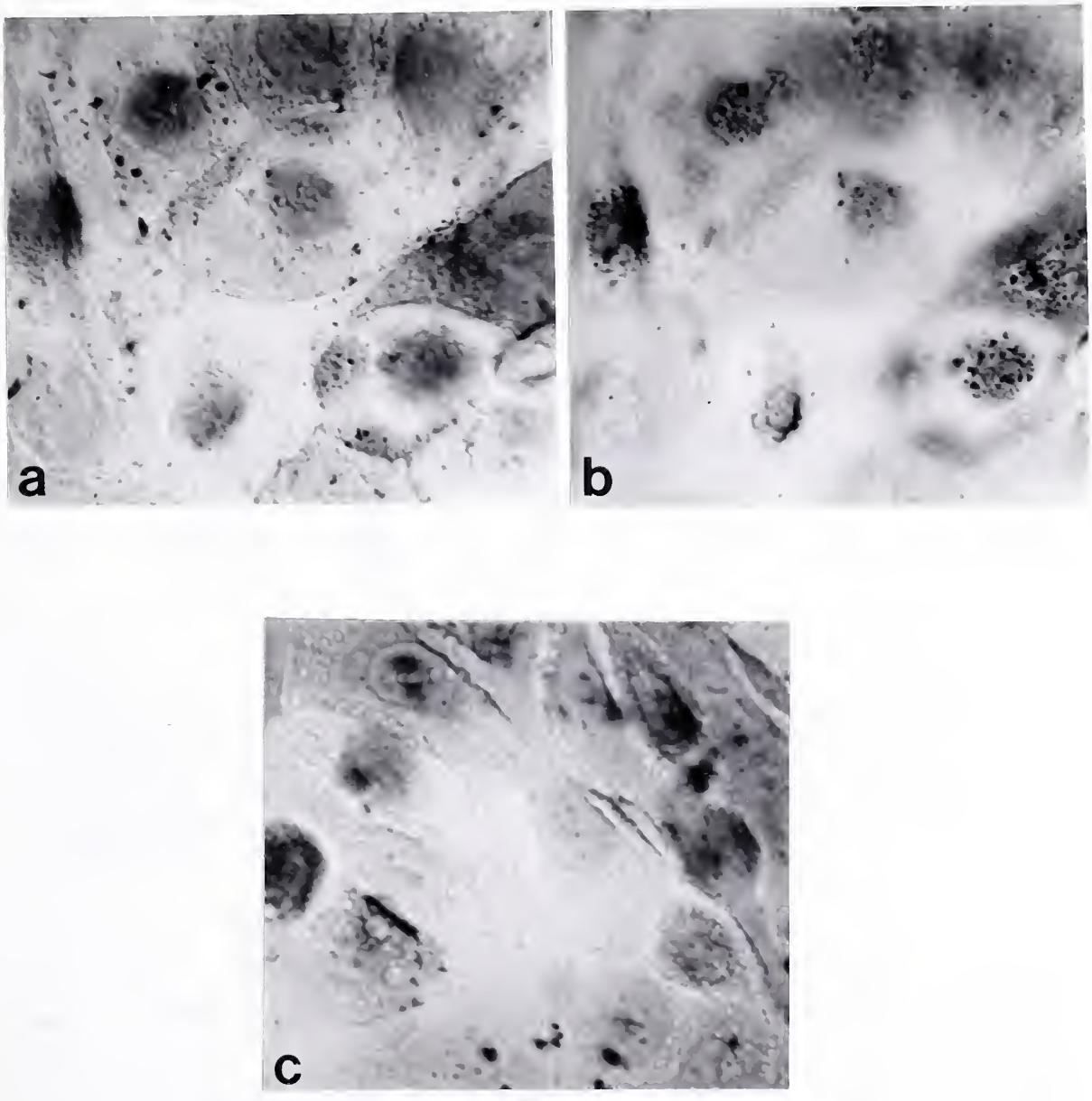

FIG. 4. Immunolocalization of CA 125 in OVCA 433 cells treated with 1 X 10-6M dexamethasone or cortisol. OVCA 433 cells stained for OC 125-binding sites and photographed as described in FIG. 3. Cells exhibit stereotypic glucocorticoid-induced morphological alterations including enlargement, increased cytoplasmic: nuclear ratio, and polygonal growth. Note granular DAB/Ni immunoprecipitate is restricted to perinuclear region;(a) focal plane 1 (b) focal plane 2 with decreased cell surface staining for CA 125 relative to untreated OVCA 433 cells in FIG. 3. (c) OVCA 433 cells treated with the physiologic glucocorticoid, cortisol, display similar pattern of staining.

Absorbance 600nm

Days	Control	10-6M	10-7M	10-8M	10-9M	10-10M
1	.063	.058	.049	.047	.044	.047
2	.113	.096	.097	.097	.105	.108
3	.232	.195	.191	.201	.206	.214
4	.306	.239	.257	.267	.287	.296
5	.426	.291	.355	.381	.422	.420
6	.602	.559	.575	.606	.574	.438
7	.592	.583	.597	.638	.586	.398

Table 1.1. Glucocorticoid inhibition of OVCA 433 cell growth. OVCA 433 cells were freshly plated with dexamethasone in concentrations ranging from 10-6M to 10-10M. Cell growth microculture assay results reported as Absorbance at 600 nm at 1-7 days post plating as described in Materials and Methods.

Log (Absorbance 600 nm x 1000)

Days	10-6M	10-7M	10-8M	10-9M	10-10M	Control
1	1.76	1.69	1.67	1.64	1.67	1.80
2	1.98	1.99	1.99	2.02	2.03	2.05
3	2.29	2.28	2.30	2.31	2.33	2.36
4	2.38	2.41	2.43	2.46	2.47	2.49
5	2.46	2.55	2.58	2.63	2.62	2.63
6	2.75	2.76	2.78	2.76	2.64	2.78
7	2.77	2.78	2.80	2.77	2.60	2.77

Table 1.2. Logarithmic representation of glucocorticoid inhibition of OVCA 433 cell growth. Cell growth microculture assay results reported as log (Abs_{600nm} X 1000) at 1-7 days post plating.

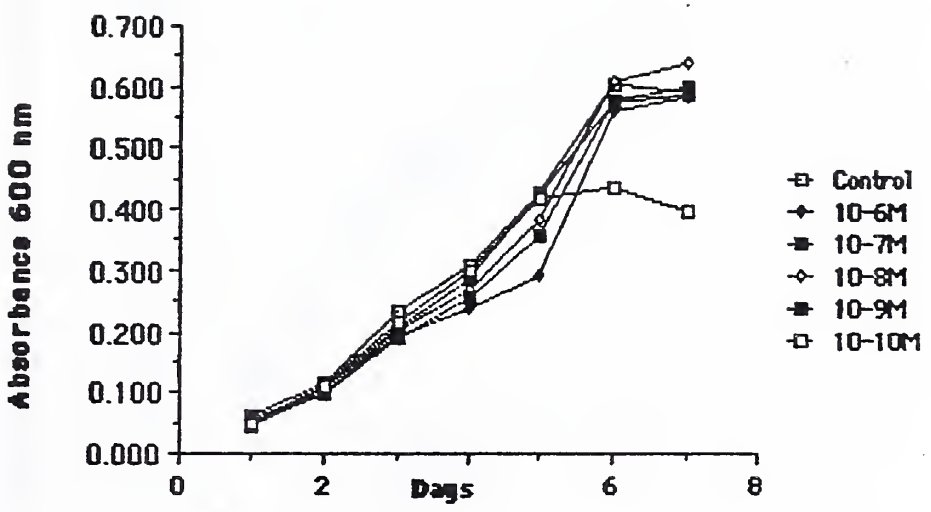

FIG. 5. Dexamethasone-induced growth inhibition of OVCA 433 cells. Abs_{600nm} vs. day in culture.

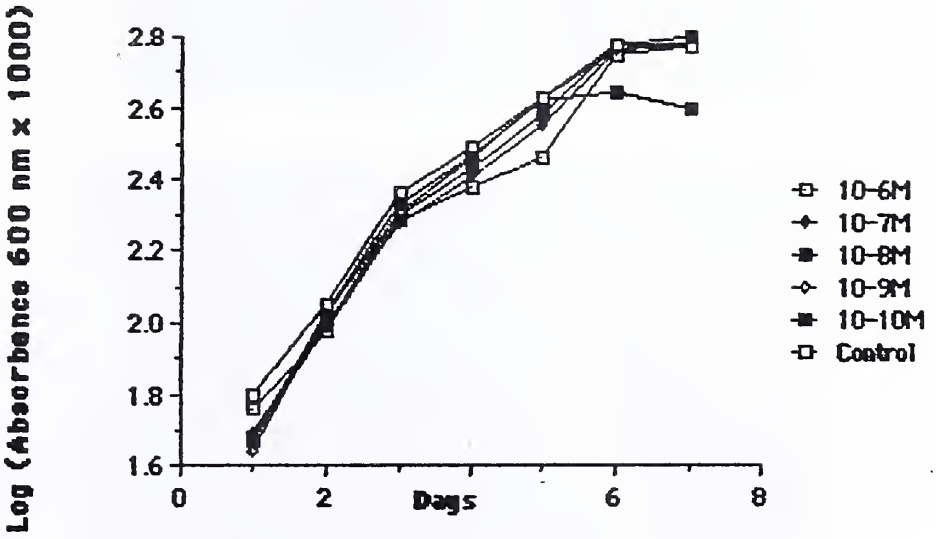

FIG. 6. Dexamethasone-induced growth inhibition of OVCA 433 cells. Log (Abs_{600nm} X 1000) vs. day in culture.

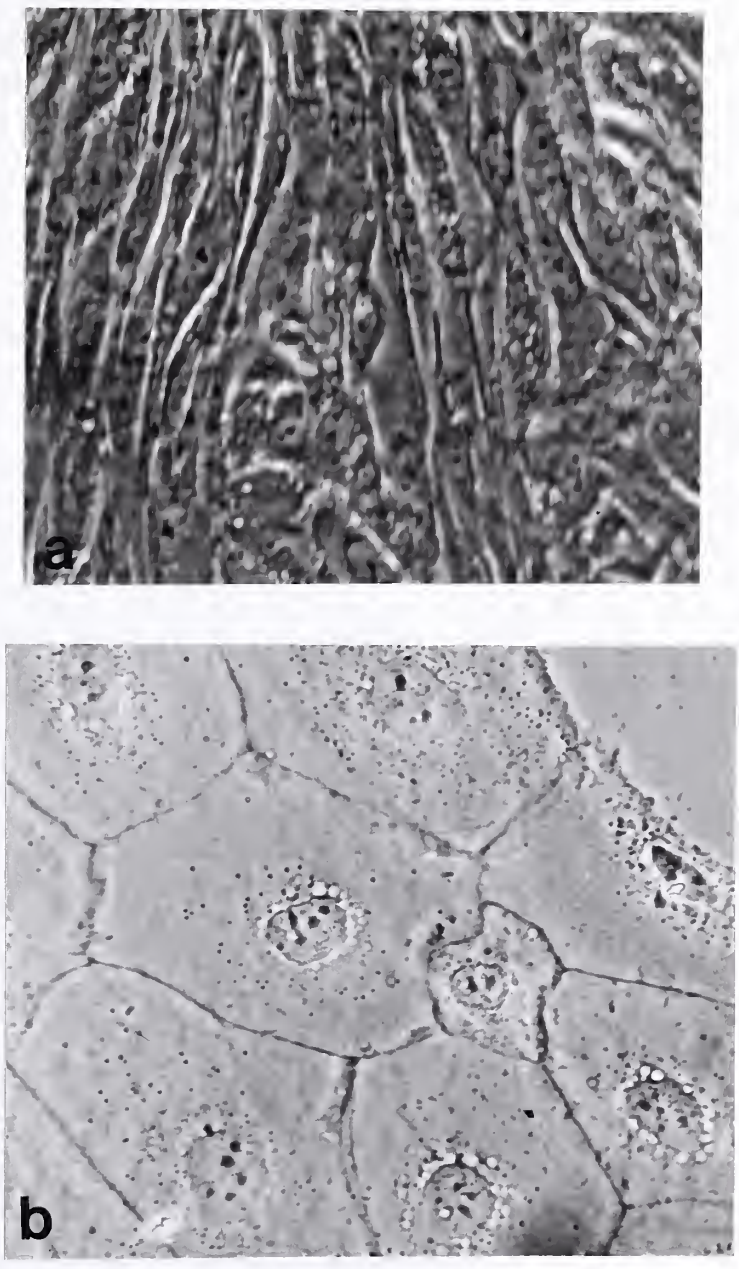

FIG. 7. Effect of OC 125 on OVCA 433 cells in vitro: Altered morphology and pattern of growth. OVCA 433 cells were plated in (a) MEM, 10% FCS or (b) MEM, 10% FCS + 30 ug/ml OC 125. Note dramatic cell enlargement, increased cytoplasmic:nuclear ratio, and neat, hexagonal array of nonoverlapping cells in (b).

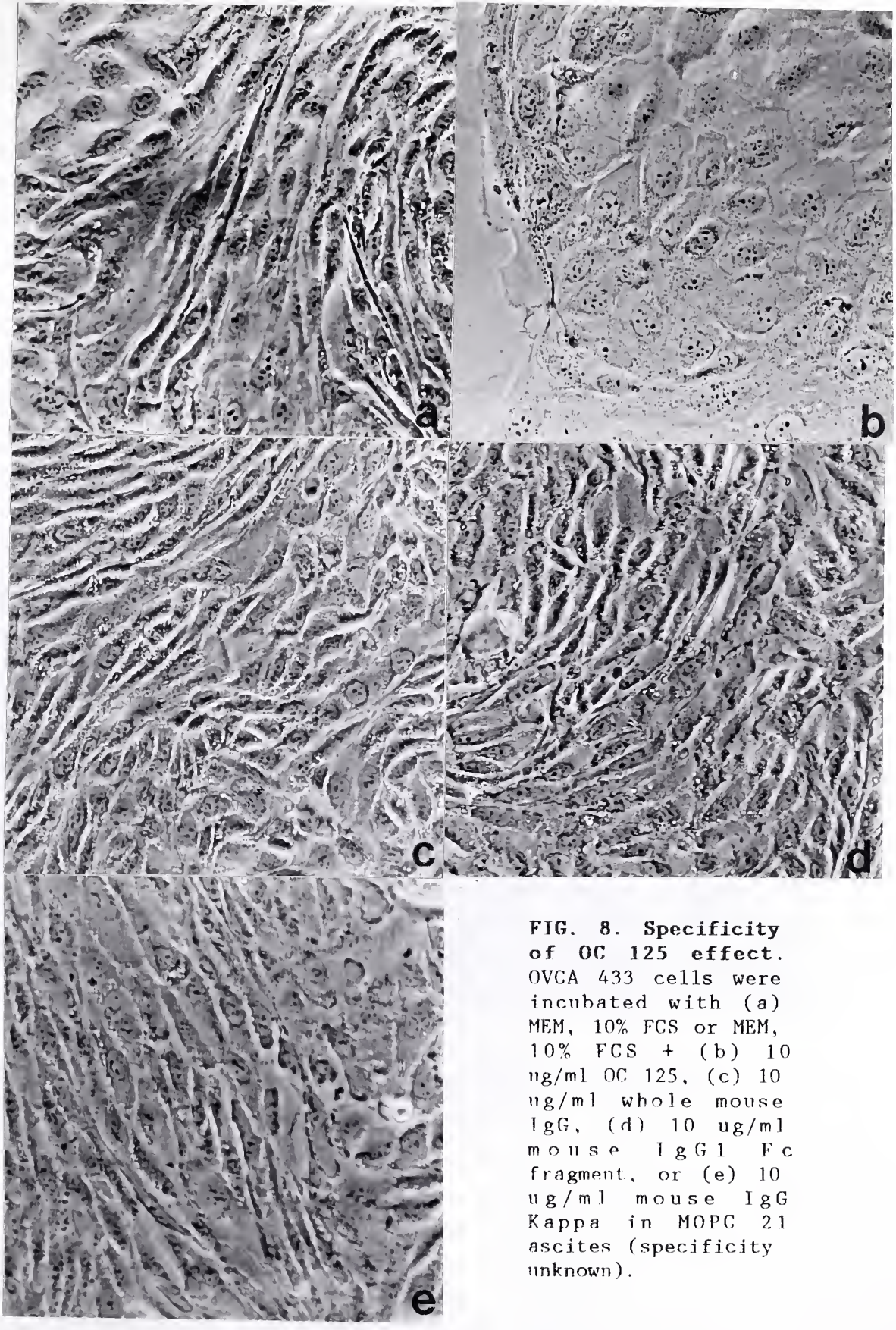

FIG. 8. Specificity of OC 125 effect. OVCA 433 cells were incubated with (a) MEM, 10% FCS or MEM, 10% FCS + (b) 10 ug/ml OC 125, (c) 10 ug/ml whole mouse IgG, (d) 10 ug/ml mouse IgG1 Fc fragment, or (e) 10 ug/ml mouse IgG Kappa in MOPC 21 ascites (specificity unknown).

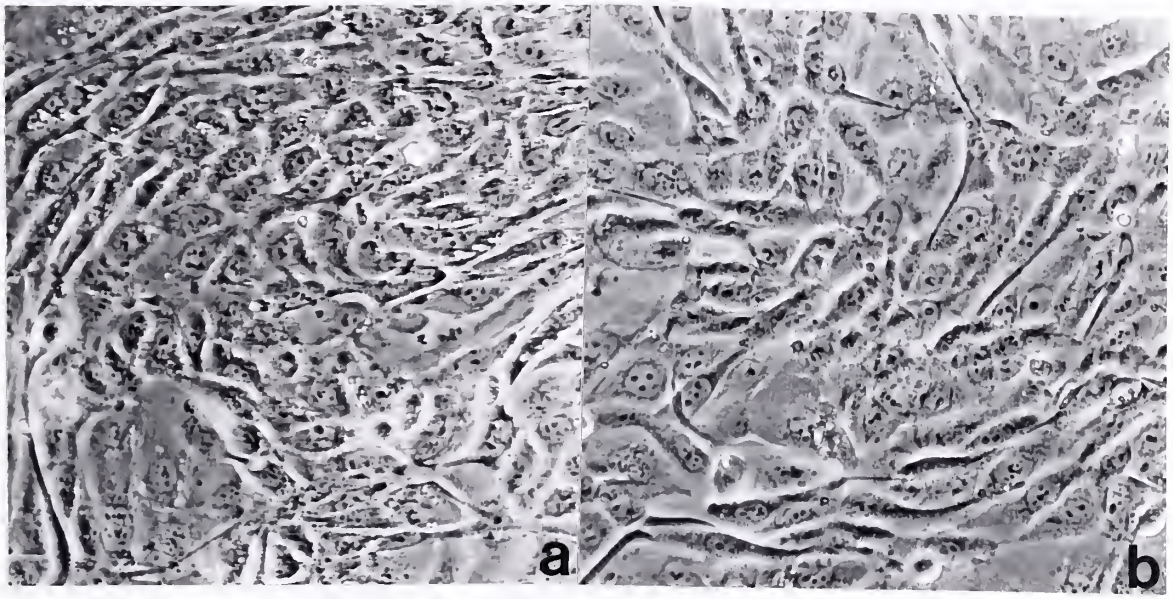

FIG. 9. Specificity of OC 125 effect. OVCA 433 cells were plated in MEM, 10% FCS + 10 ug/ml (a) MAb anti-HCG (beta fragment) and (b) MAb anti-tissue plasminogen activator without effect.

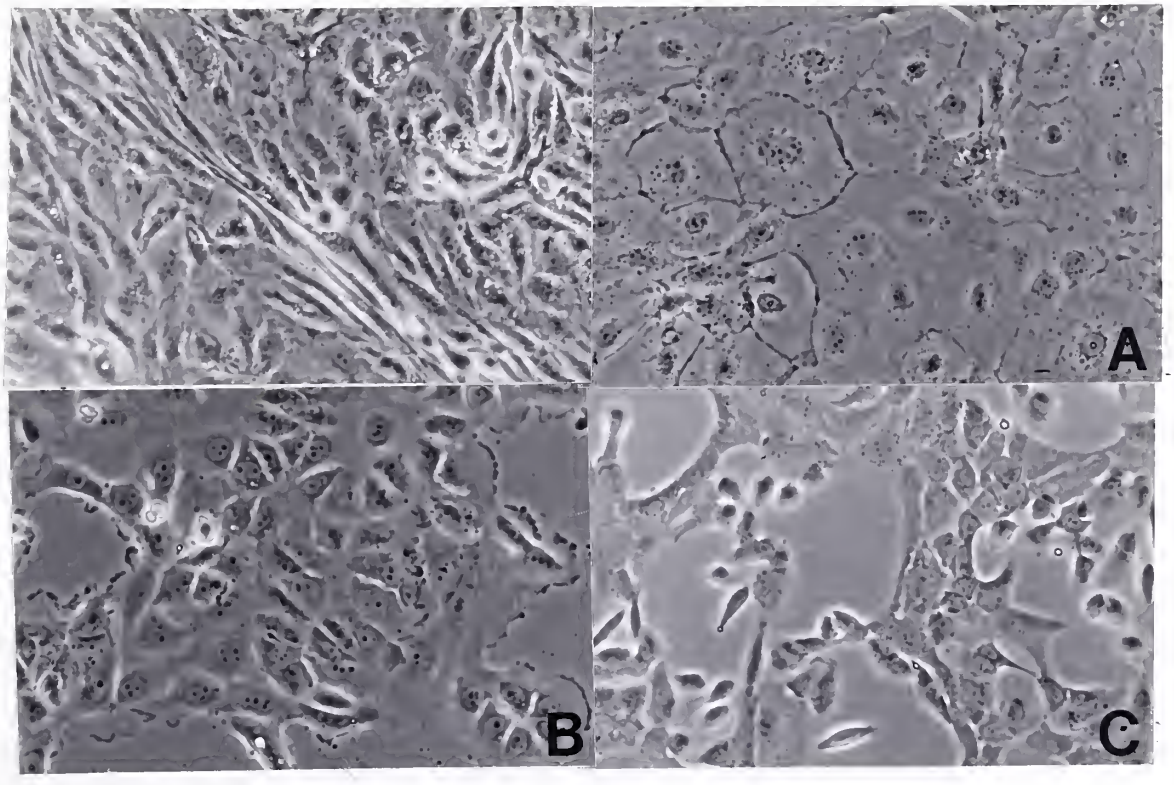

FIG. 10. Specificity of OC 125 effect.(a) OVCA 433 cells and two alternate human ovarian carcinoma cell lines,(b) Caov-3 and (c) SK-OV-3, which do not express significant quantities of CA 125 were plated in MEM, 10% FCS + 10 ug/ml OC 125. Only OVCA 433 cells exhibit altered morphology. Littlefield previously demonstrated that OVCA 433 cells secrete >1000 U/ml CA 125 into the culture media while antigen is not detectable in supernatants harvested from Caov-3 and SK-OV-3 cultures.

OC125 INHIBITION OF OVCA 433 CELL GROWTH

n=7

[OC125] ug/ml	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
0	.063 (.006)	.113 (.003)	.232 (.005)	.308 (.004)	.426 (.011)	.602 (.014)	.592 (.012)
0.1	.052 (.003)	.106 (.003)	.218 (.004)	.239 (.013)	.396 (.035)	.650 (.017)	.639 (.019)
0.3	.046 (.002)	.106 (.003)	.229 (.010)	.311 (.009)	.467 (.013)	.649 (.018)	.624 (.018)
1.0	.051 (.003)	.106 (.002)	.221 (.004)	.308 (.008)	.453 (.010)	.579 (.004)	.559 (.022)
3.0	.053 (.004)	.103 (.003)	.213 (.006)	.296 (.007)	.397 (.015)	.418 (.008)	.370 (.014)
10.0	.048 (.003)	.095 (.003)	.186 (.005)	.243 (.005)	.317 (.012)	.011 (.003)	.014 (.005)
30.0	.047 (.004)	.073 (.004)	.108 (.003)	.115 (.004)	.122 (007)	.176 (.004)	.158 (.005)

Table 2.1. OC 125 inhibition of OVCA 433 cell growth. OVCA 433 cells were freshly plated with OC 125, anti-CA 125 MAb, at concentrations of 0, 0.1, 0.3, 1.0, 3.0, 10.0, and 30.0 ug/ml. Cell growth microculture assay results reported as Absorbance at 600nm +/- SE at 1-7 days post plating.

OC125 INHIBITION OF OVCA 433 GROWTH

N=7 LOG (1000 X Abs 600 nm)

[OC12] ug/ml	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
0	1.80	2.05	2.36	2.49	2.63	2.78	2.77
0.1	1.72	2.03	2.34	2.38	2.60	2.81	2.81
0.3	1.66	2.03	2.36	2.49	2.67	2.81	2.80
1.0	1.71	2.03	2.34	2.49	2.66	2.76	2.75
3.0	1.72	2.01	2.33	2.47	2.60	2.62	2.57
10.0	1.68	1.98	2.27	2.39	2.50	1.04	1.15
30.0	1.67	1.86	2.03	2.06	2.09	2.25	2.20

Table 2.2. Logarithmic representation of OC 125-induced growth inhibition of OVCA 433. Cell growth microculture assay results reported as log(Abs_{600nm} X 1000) at 1-7 days post plating.

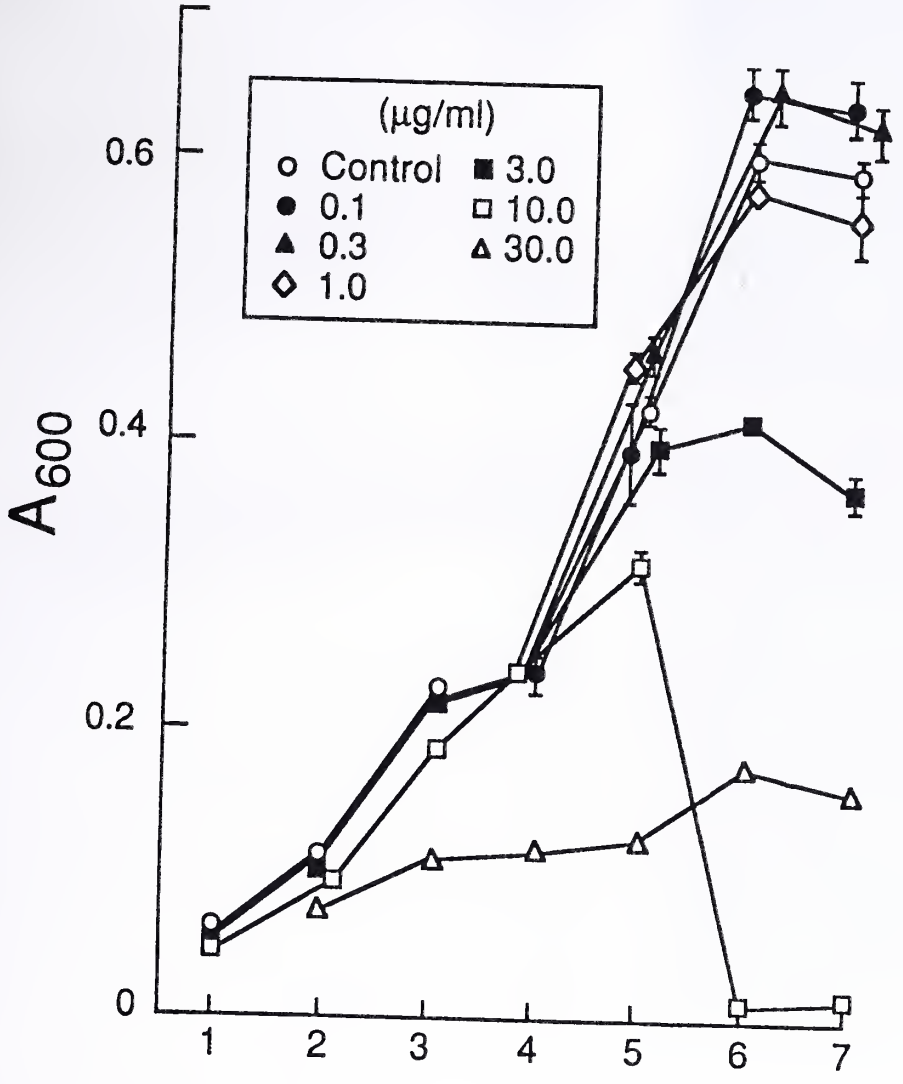

FIG. 11. OC 125 growth inhibitory effect Abs_{600nm} vs. day in culture

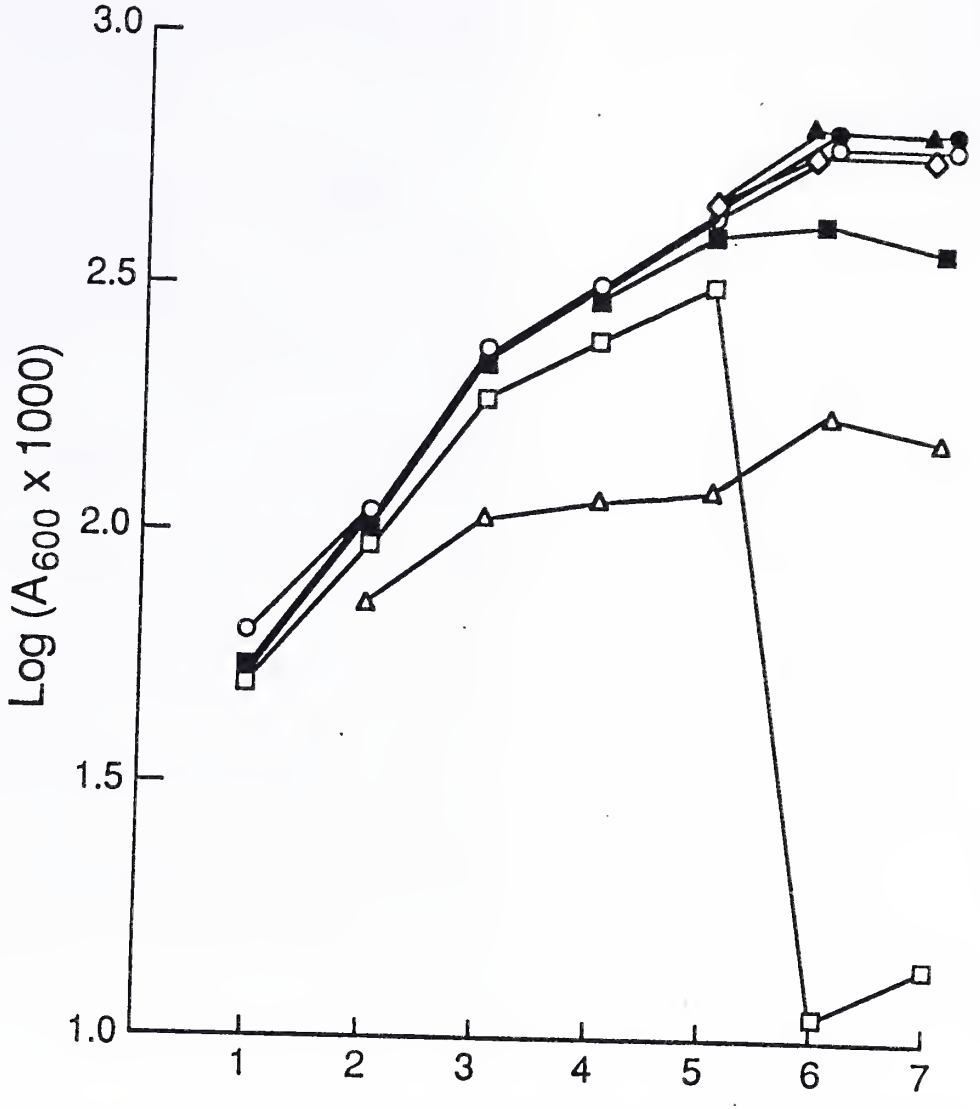

FIG. 12. OC 125 growth inhibitory effect on OVCA 433 cells. Log (Abs_{600nm} X 1000) vs. day in culture.

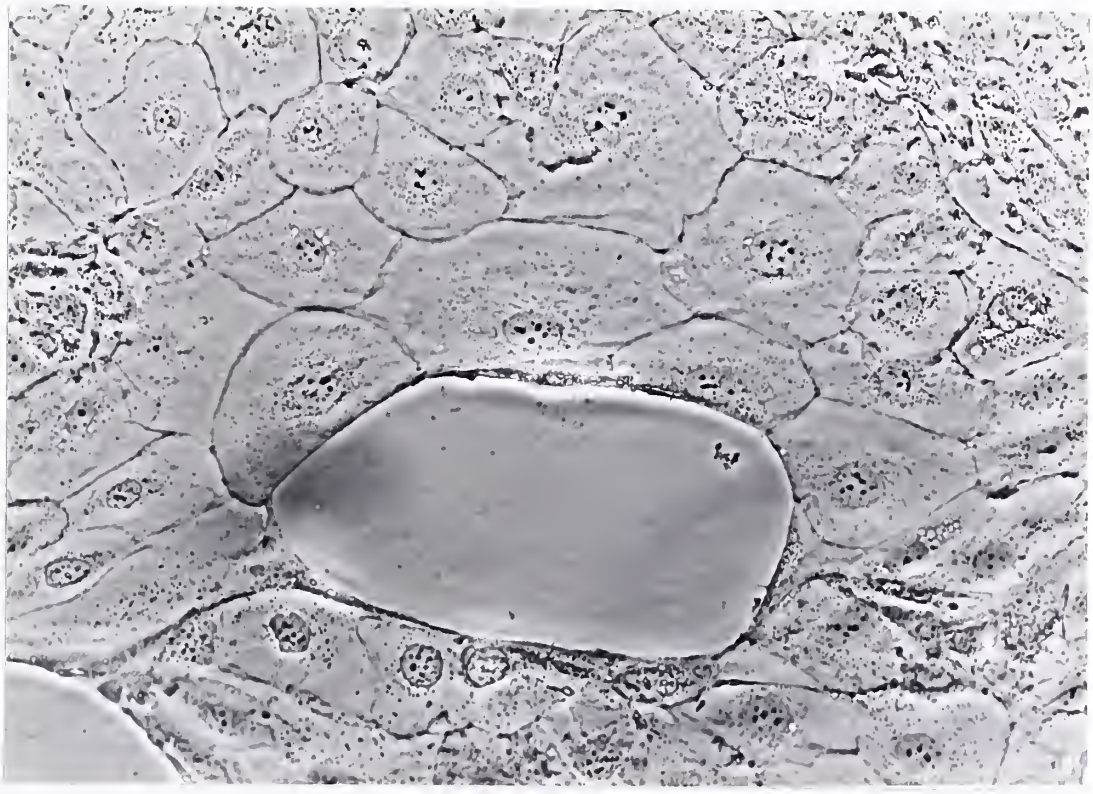

FIG. 13. OVCA 433 cells freshly plated with 30 ug/ml OC 125 after 4 days in culture begin to lift off plastic forming structures reminiscent of the lining epithelial cells of microcysts noted in serous cystadenocarcinomas.

ANTIGENS ASSOCIATED WITH HUMAN EPITHELIAL OVARIAN CARCINOMAS DEFINED BY MONOCLONAL ANTIBODIES

ANTIGEN	MONOCLONAL ANTIBODY	INVESTIGATORS
High-molecular-weight glycoproteins >200 kd	OC 125	Bast
	NS 19-9*	Koprowski
	1D3	Bhattacharya
	MOV-2*	Tagliabue
	DUPAN-2*	Metzgar
	F 36/22*	Croghan
	DF3*	Sekine
	HMFG1,HMFG2	Epenetos
200 kd protein	454C11	Frankel
105 kd glycoprotein	MF116	Mattes
80 kd protein	OC133	Miotti
72 kd protein	791-T/36	De Groote
70 kd protein	WB12123	Knauf
60 kd protein	$2C_8,2F_7$	Bhattacharya
48 kd protein	$4F_4,7A_1{}^0$	Bhattacharya
Low-molecular-weight glycolipids	NS 19-9*	Koprowski
	MOV-2*	Tagliabue

*Carbohydrate determinants

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